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Animal health and greenhouse gas intensity: the paradox of periparturient parasitism

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Abstract

Here we provide the first direct measurements of pathogen challenge impacts on greenhouse gas (GHG) production, yield and intensity. Twin-rearing ewes were *ad libitum* fed pelleted lucerne from day_{-32} to day_{36} (day_0 is parturition), and repeatedly infected with 10,000 *Teladorsagia circumcincta* infective larvae (n=16), or sham-dosed with water (n=16). A third group of 16 ewes were fed at 80% of uninfected ewes' feed intake during lactation. Methane emissions were measured in respiration chambers (day_{30} to day_{36}) whilst total tract apparent nutrient digestibility around day_{28} informed calculated manure methane and nitrous oxide emissions estimates. Periparturient parasitism reduced feed intake (-9%) and litter weight gain (-7%) and doubled maternal body weight loss. Parasitism reduced daily enteric methane production by 10%, did not affect methane yield per unit dry matter intake but increased yield per unit digestible organic matter intake by 14%. Parasitism did not affect daily calculated manure methane and nitrous oxide production, but increased manure methane and nitrous oxide yields per unit dry matter intake by 16% and 4%, respectively, and per unit digestible organic matter intake by 46% and 31%, respectively. Accounting for increased lucerne input for delayed weaning and maternal body weight loss compensation, parasitism increased calculated GHG intensity per kg lamb weight gain for enteric methane (+11%), manure methane (+32%) and nitrous oxide (+30%). Supplemented with the global warming potential (GWP) associated with production of pelleted lucerne, we demonstrated that parasitism increased calculated GWP per kg lamb weight gain by 16%, which was similar to the measured impact of parasitism on feed conversion ratio. Thus, arising from

pathogen-induced feed efficiency reduction and modified GHG emissions, we demonstrated that ovine periparturient parasitism increases GHG intensity. This implies that ewe worm control can not only improve production efficiency but also reduce the environmental footprint of sheep production systems.

Key words: disease, parasitism, environmental footprint, methane, nitrous oxide, sheep

1. Introduction

It is well recognized that pathogen exposure often results in anorexia, i.e. reduction in feed intake. In the case of sub-clinical gastrointestinal nematode parasitism, feed intake is typically reduced by up to 20-25% in e.g. growing and periparturient sheep, though wide ranges of parasitism-induced anorexia and associated production losses have been reported across different species (Sykes, 1994; Kyriazakis et al., 1998; Zaralis et al., 2008). Variation in feed intake can be expected to correlate with variation in greenhouse gas (GHG) production from both respiration and manure emission. This implies that pathogen challenge would be expected to result in reduced daily production of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O), provided that GHG yield, defined as the amount of GHG produced per unit feed intake, is not affected. However, since pathogen challenge reduces productivity, arising from a combination of anorexia and reduced efficiency of resource use for production purposes (Sykes, 1994; Coop and Kyriazakis, 1999), challenged animals would be expected to take longer and require more resource input to achieve the same productive output. GHG production associated with this extra resource input required would effectively be the consequence of pathogen challenge on resource efficiency, and thus increase GHG intensity.

Here, we provide the first direct assessment of the impact of pathogen challenge on GHG emission in livestock. We have assessed effects of gastrointestinal parasitism on performance, digestibility, CH_4 and CO_2 production and yield, and on feed efficiency, in lactating ewes. Furthermore, we used IPCC (2006) assumptions and literature data where data were not

derived from the experiment carried out to extend the above to include estimates of manure CH_4 and N_2O production and yield. Theses estimates are used to test the hypothesis that periparturient parasitism increases ewe GHG intensity and global warming potential (GWP) for lamb production.

2. Materials and Methods

2.1 Animals and housing

Twelve 4-5 year old Mule ewes (Bluefaced Leicester × Scottish Blackface) were recruited from each of four larger mating group approximately 45 days before observed mean parturition date (day_0), with mean expected parturition dates separated by a week. Ewe body weight (BW) and body condition score (CS) recorded on day_{-39} for the total of 48 ewes used averaged (±se) 68.2±0.79 kg and 2.5±0.06, respectively. Ewes were served by Suffolk rams and confirmed to be bearing twins by ultrasonic scanning prior to the experiment and were housed individually, in pens sized 1.30 m × 2.15 m with adjacent creep area of the same size for their lambs. From day_{-45} until day_{30}, the ewes were housed in a naturally ventilated and illuminated shed, with additional low-level lighting at night times during lambing. From day_{30} until day_{36}, ewes and their lambs were housed in respiration chambers (see below) in similar sized pens. Fresh wood shavings were used as bedding and added daily, and fresh water was *ad libitum* available. A small amount of shavings were also used daily in the respiration chambers.

2.2. Experimental treatments and feeding

98 The twelve ewes within each of the four mating groups were divided
99 into three groups of four ewes based on initial BW, which resulted in three
100 groups of 16 ewes with similar mean initial BW, CS and faecal egg counts
101 (FEC). From day$_{-45}$ to day$_{-32}$, the ewes received *ad libitum* medium quality hay
102 and approximately 300 g/day/head of a commercial ewe nut. From day$_{-32}$ until
103 day$_{-25}$, allowances of hay and commercial ewe nuts were gradually reduced
104 and completely replaced with increasing amounts of pelleted lucerne. From
105 day$_{-24}$ onwards, two groups of ewes were fed lucerne *ad libitum* and either
106 uninfected (CON) or dosed with parasites (PAR). Details of the experimental
107 infection are provided below. A third group of ewes were managed as CON
108 ewes during pregnancy but fed restrictedly at 80% of intakes achieved by
109 CON ewes during lactation (RES). The RES group was included to assess to
110 what extent GHG production, yield and intensity would be affected by reduced
111 feed intake per se. Ewes were fed at 07.30 and 1500 h. The experiment was
112 approved by SRUC's Ethical Review Committee (ED AE 03/2011) and carried
113 under Home Office authorization (PPL 60/3782).

114

115 **2.3. Experimental infection**

116 Because the ewes were 4 to 5 years old and had previously grazed
117 natural pastures infested predominantly with *Teladorsagia circumcincta*, they
118 were expected to have had substantial prior exposure to this parasite, an
119 abomasal nematode of particular concern in temperate regions. The ewes
120 were orally treated on day$_{-38}$ with levamisole (Levacide, Norbrook, Newry, UK)
121 and ivermectin (Oramec, Merial, Harlow, UK) at the rate of 7.5 and 0.2 mg/kg
122 BW, respectively, to remove worm burdens. A subsequent FEC taken on day-

$_{22}$ averaged 0 (0-1) eggs per g fresh faeces (epg), suggesting that the drench was effective. The PAR ewes were then trickle infected with 10,000 infective *T. circumcincta*, suspended in 10 ml of water and administered every Monday, Wednesday and Friday from day$_{-21}$ onwards until the end of the experiment. The CON and RES ewes were sham-infected with 10 ml of water on the same days. The *T. circumcincta* strain used was the Moredun Ovine Susceptible Isolate that has been maintained in the laboratory for several years. This infection model has repeatedly been used in our lab to induce sub-clinical parasitism in periparturient ewes (Houdijk et al., 2003, 2006, Zaralis et al., 2009; Kidane et al., 2010).

2.4. Measurements and calculations

Performance. The ewes were weighed on day-39 and then weekly from day-31 onwards, as well as within 12 h of parturition to assess daily weight gain during late pregnancy and during lactation through linear regression of BW on time. The lambs were weighed within 12 h after birth and weekly afterwards to assess daily litter weight gain in the same way. Since the lambs did not receive creep feed, lamb BW and daily weight gain were used to calculate milk production (Robinson et al., 1969). Ewe CS was taken approximately fortnightly, by lumbar palpation on a zero to five point scale, and to an accuracy of a quarter (Russel et al., 1969), where 0 is emaciated and 5 is obese. Feed samples were collected every day during the experiment during feeding and were pooled for chemical analyses (Table 1) as per standard protocols (Ministry of Agriculture Fisheries and Food, 1992). Feed refusals were recorded twice weekly (Mon and Thu) and analysed for dry

matter (DM) only. This allowed for the calculation of achieved mean daily dry matter intake (DMI).

Parasitism. The level of parasitism was monitored through regular faecal sampling for FEC, according to a modified flotation method (Christie and Jackson, 1982), with a sensitivity of 1 epg. This was done for all ewes at housing, day-22 and day-11 and at parturition, and then weekly thereafter (for PAR ewes only).

Digestibility. Apparent total tract DM, organic matter (OM) and nitrogen (N) digestibility were assessed through using acid insoluble ash (AIA) as an internal, indigestible marker. Feed samples collected daily during feeding were pooled for DM, N, ash and AIA analyses, with OM calculated as DM minus ash. Faeces were collected directly from the rectum of all ewes for three consecutive days (day_{27} to day_{29}) and were pooled per individual ewe and kept frozen at -20 ^{0}C before analysis of DM, ash, AIA and N, and calculation of OM as above. Feed and faecal AIA were analysed using the 2 M HCl procedure described by van Keulen and Young (1977). The above analyses allowed us to calculate faecal OM and N output, as well as digestible OM intake (dOMI).

Furthermore, daily fresh faeces production was calculated using mean achieved DM intake, faeces DM contents and total tract DM digestibility in order to estimate worm egg output (eggs/day). The latter was estimated by multiplying mean FEC during lactation (eggs/g) with the calculated fresh faeces production (g/day), under the assumption that since ewes were fed the same diet throughout lactation, total tract DM digestibility measured from

171 day_{23} to day_{26} can be extrapolated over the whole lactation period (Kidane et
172 al. 2009).

173 ***Enteric methane and carbon dioxide emissions.*** Staggered lambing
174 arising from the four mating groups used allowed for four rounds of housing in
175 one of six indirect open-circuit respiration chambers (No Pollution Industrial
176 Systems Ltd., Edinburgh, UK) for six days from day_{30} to day_{36} with two ewes
177 per treatment per chamber, individually housed with their lambs to achieve
178 two treatment replicates per round, and thus n=8 in total. Experimental
179 treatments and DMI determination were maintained in chamber. Daily CH_4
180 and CO_2 production was measured as described in detail elsewhere (Rooke
181 et al., 2014). Briefly, each chamber has an area of 25.4 m^2 with appropriate
182 penning for two ewes and their four lambs. Air was removed from the
183 chambers by exhaust fans set at 50 litre/s and temperature and humidity were
184 set at 15 ± 1°C and 60 ± 5% relative humidity, respectively. Exhaust air was
185 sampled for gas analysis sequentially for 45 s from each chamber, and
186 methane and carbon dioxide concentrations were measured by infrared
187 absorption spectroscopy. Animals remained in the chambers for 6 days,
188 where the first four days were used for adaptation, and the last two days were
189 used to quantify CH_4 and CO_2 production. Measurements of CH_4 and CO_2
190 concentrations were made every 6 minutes in the mechanically ventilated air
191 entering and leaving each chamber and exhaust air flow rate (every 30 min)
192 corrected to standard temperature and pressure. Due to missing data arising
193 from operational issues in Round 2, a full data set for each room was
194 available for only the last 24 h, which was the dataset used for the present

195 analysis. Daily CH_4 and CO_2 production was divided by mean daily DMI and
196 dOMI to obtain CH_4 and CO_2 yield.

197 ***Manure methane emissions*.** Methane emissions from manure were
198 estimated from the volume of volatile solids produced, defined as the sum of
199 faecal and urine OM output (IPCC, 2006). Faecal OM output was derived from
200 mean daily in chamber DMI, feed OM content and total tract OM digestibility,
201 whilst total urine OM output was calculated under the assumption that energy
202 excretion in urine is 0.04 × GE intake (IPCC, 2006) and that energy
203 concentration in urine is 18.75 MJ/kg DM (IPCC, 2006). We further assumed
204 that the manure is directly deposited on pasture in a cool climate (i.e. not
205 stored), and thus a methane conversion factor of 1% and a maximum
206 methane-producing capacity of 0.19 m^3 per kg volatile solids (IPCC, 2006).
207 This resulted in a methane yield of 1.273 g per kg volatile solids. Daily manure
208 CH_4 production was divided by mean daily in chamber DMI and dOMI to
209 obtain manure CH_4 yield estimates.

210 ***Manure N_2O emissions.*** The N_2O emission from manure was
211 estimated from total N excreted, i.e. the sum of faecal N and urine N outputs.
212 Faecal N output is derived from mean daily in chamber DMI, feed N content,
213 and apparent total tract N digestibility. Urine N output is estimated from the
214 assumption that urine N output equals 0.46 × N apparently absorbed during
215 lactation. This coefficient was derived from a series of then N balance
216 estimates in lactating ewes (Lynch et al., 1988; Malik et al., 1999; Pappas,
217 1977; Maamouri et al., 2011), whilst N absorbed was derived from apparent
218 total tract N digestibility carried out. As above, we assume that the manure is
219 deposited directly on the pasture in a cool environment, and 1% of manure N

is directly volatised into nitrous oxide N, 20% of manure N is indirectly volatilised through ammonia with an efficiency factor of 0.01 for conversion into nitrous oxide N and 30% of manure N is leached through nitrate with an efficiency factor of 0.0075 for conversion of manure N into nitrous oxide N (IPCC, 2006). This resulted in a N_2O yield estimate of 22.393 g per kg N excreted. Resulting daily N_2O production was divided by mean daily DMI and dOMI to obtain N_2O yield.

Greenhouse gas emission intensity calculations. The GHG yields derived as above were used to calculate GHG emission intensity per functional unit, which was one kg lamb BW gain (BWG) from parturition until weaning. We used the observed DMI, ewe BW loss, lamb birth weight and lamb BW gain until day_{36}, to calculate through extrapolation the number of days and the amount of DMI needed, as well as the total ewe BW loss incurred, to reach a target weaning weight of 25 kg live weight for each of two lambs reared (Kidane et al., 2010). This DMI was multiplied with GHG yields as measured for CON, PAR and RES ewes.

We also calculated DMI needed to restore final ewe BW to initial ewe BW (day-39), to account for BW loss incurred as a resource used to rear lambs to weaning. We assumed that the metabolizable energy (ME) requirement for restoring ewe BW was 39.75 MJ/kg for CON and RES ewes (AFRC, 1993) but 49.16 MJ/kg for PAR ewes for reasons outlined below (see 'sensitivity analysis'). Since any influence of PAR or RES treatment on digestibility and efficiency of resource utilization for weight gain post weaning would unlikely remain present post weaning, as ewes return to full immunity to parasites when lactation ceases (Houdijk, 2008), DMI to restore ewe BW was

multiplied with mean GHG yields for CON ewes. Total DMI was divided over total lamb weight gain to calculate feed conversion ratio. Furthermore, post weaning urine N output was estimated from the assumption that urine N output equals 0.76 × N apparently absorbed; this coefficient was derived from a series of then N balance estimates in growing sheep (Akinbamijo et al., 1994; Lima et al., 2011; Gorniak et al., 2014; Kimambo et al., 1988).

GHG emission intensity data from the above described CH_4 and N_2O sources were combined into one GWP figure after converting CH_4 and N_2O into their CO_2 equivalents. Conversion factors used were 25 and 298 CO_2-eq for methane and nitrous oxide, respectively (IPCC, 2006). Data from CH_4 and N_2O were supplemented with a GWP of 320 g CO_2-eq/kg for the production, dehydration and transport of lucerne with 11% residual moisture (Gallego et al., 2011), to derive at total GWP (kg CO_2-eq/kg BWG). Thus, breath CO_2 was not included in these calculations, as GWP assessments focus on net contributors to global warming, i.e. fossil-derived CO_2 only. We also used lamb BWG to estimate milk production (Robinson et al., 1969), in order to estimate GHG intensity per kg milk.

Sensitivity analysis. It was deemed appropriate to use a greater requirement of ME per kg BW gain to restore BW for PAR ewes than for CON and RES ewes, as PAR ewes likely lost relatively more fat than did CON and RES ewes (see results on CS). The 49.16 MJ/kg BW gain was arbitrarily chosen as the average of 39.75 MJ/kg (AFRC, 1993) and 58.57 MJ/kg (Olthoff et al. 1989), which would be requirements for fat deposition only. A sensitivity analysis was undertaken for the impact of this assumption by

comparing GWP intensity for PAR ewes to that of CON and RES ewes for ME requirements per kg BW gain taken as 39.75, 49.16 and 58.57 MJ/kg.

Since there are no data on the effect of parasitism in general, and of *T. circumcincta* challenge in particular, on urine N excretion in lactating sheep, we assumed that the aforementioned urine N as a proportion of apparent N absorbed is similar between CON, PAR and RES ewes. However, earlier studies showed that *T. circumcincta* infections in young lambs increased urinary N output as a proportion of apparent N absorbed by 30 to 40% relative to non-infected control lambs (Parkins et al., 1973; Sykes and Coop, 1977). We therefore undertook a second sensitivity analysis by comparing GWP intensity for urine N output by increasing the value of 0.46 × N absorbed to 0.65 (40% increase) during lactation.

2.5. Statistical analysis

Data obtained during late pregnancy and early lactation were analysed separately, whilst the effect of parasitism during late pregnancy was analysed through comparing PAR ewes with combined CON/RES ewes. During late pregnancy, parameters that were repeatedly taken, i.e. feed intake, ewe BW and CS were analysed via repeated measures ANOVA. Parameters that were measured once, i.e. ewe and litter BW at birth, were analysed through ANOVA. Likewise, during lactation, ewe feed intake, BW, CS and hourly methane and carbon dioxide production were analysed via repeated measures ANOVA, whilst ewe and litter BW gain, digestibility figures, daily GHG production, yield and intensity data were analysed through ANOVA.

293 Models included mating group as block and day$_{-39}$ observations as
294 covariate where appropriate. The ewe was the experimental unit for
295 production, parasitological and digestibility observations (n=16). However, for
296 any parameter related to emission production, yields and intensities, the
297 respiration chamber with paired ewes was used as experimental unit (n=8).
298 Means were separated through the use of Fisher's protected LSD test at
299 $P<0.05$.
300
301 **3. Results**
302 **3.1. Performance during late pregnancy**
303 Figure 1 shows mean food intake over time during both late pregnancy
304 and early lactation. Treatment did not interact with time for DM intake during
305 late pregnancy (P=0.33), which averaged 3.02 and 2.77 kg/day for the
306 combined CON/RES ewes and PAR ewes, respectively (s.e.d. 0.13 kg/day;
307 P=0.05). Time and treatment did not interact for BW during late pregnancy
308 (P=0.70), reflected in an numerically greater BW gain for the combined
309 CON/RES ewes than for PAR ewes at 272 and 228 g/day, respectively (s.e.d.
310 31 g/day, P=0.11). CON/RES ewes tended to be heavier at parturition than
311 PAR ewes, averaging 70.1 and 68.0 kg, respectively (s.e.d. 1.19 kg; P=0.08),
312 though litter BW at birth did not differ, averaging at 9.07 and 9.30 kg,
313 respectively (s.e.d. 0.44 kg; P=0.61). Treatment did not interact with time for
314 CS (P=0.47) during late pregnancy, which gradually increased from 2.51±0.06
315 on day$_{-39}$ to 2.72±0.06 on day$_{-6}$ (P=0.017).
316

3.2. Total tract apparent digestibility and performance during early lactation

Table 2 shows that the total tract DM, OM and CP digestibilities were smaller in PAR ewes than in both CON and RES ewes (P<0.05). As for late pregnancy, feed intake and time did not interact during early lactation (Figure 1; P=0.33). Table 2 shows that mean DM intake was greater for CON ewes than for PAR ewes, which was in turn greater than for RES ewes. However, PAR and RES ewes achieved similar levels of dOM and digestible CP intake, which were both smaller than those for the CON ewes. Ewe BW loss was less for CON ewes than for PAR ewes, which in turn was less than for RES ewes. However, litter BW gain and calculated milk production were greater for CON ewes than for PAR and for RES ewes, whilst both did not differ between PAR and RES ewes (Table 2).

Treatment tended to interact with time for CS (P=0.07) during lactation; CS averaged 2.46±0.06 across treatments on day_{6} and 2.08, 1.84 and 2.02 on day_{29} for CON, PAR and RES, respectively (se 0.07), suggesting that CS reduced for all ewes (P<0.01) but at a higher rate for PAR from ewes than for CON and RES ewes during lactation.

3.3. Parasitism

Ewe FEC averaged 101 (86 - 119) epg on day_{-39} and 0 (0 - 1) epg at day_{-22}. Following infection from day-21 onwards, FEC of CON/RES and PAR ewes averaged 1 (1-2) and 0 (0-1) epg on day_{-11}, respectively, and 3 (2-4) and 26 (15-43) epg at parturition, respectively (P<0.001). The FEC of PAR ewes then gradually increased to 71 (48 to 106) epg by day_{28}. Combined with

342 calculated fresh faeces production, the latter translated into 962 (675-1371) x
343 10^3 worm eggs per day.
344
345 **3.4. GHG production and yield**
346 Figure 2 displays the average hourly CH_4 (Fig 2a) and CO_2 (Fig 2b)
347 production of sets of two CON, PAR and RES ewes. Experimental treatment
348 interacted with time for both GHG ($P<0.001$), arising from the larger diurnal
349 variation in the RES ewes. Table 3 shows enteric and manure GHG
350 production and yield per kg dry matter intake (DMI) as well as per kg
351 digestible organic matter intake (dOMI), expressed both as CH_4 and N_2O and
352 converted to CO_2 equivalents. We observed that CON ewes produced more
353 enteric CH_4 than PAR ewes, which in turn produced more enteric CH_4 than
354 RES ewes. However, whilst enteric CH_4 yield per kg DMI did not differ,
355 averaging 10.23 g per kg DMI, enteric CH_4 yield per kg dOMI was ~14%
356 greater for PAR ewes than for CON and RES ewes ($P<0.05$). We observed
357 similar patterns for CO_2 emissions, though CO_2 production did not differ
358 between CON and PAR ewes ($P=0.15$), CO_2 yield per kg DMI did not differ,
359 averaging 690 g, whilst CO_2 yield per kg dOMI for RES ewes was
360 intermediate to that of CON and PAR ewes, whose CO_2 yields differed
361 ($P<0.05$).
362 The pattern in manure GHG emissions differed from enteric GHG
363 emissions. CON and PAR ewes produced similar volumes of manure volatile
364 solids, and thus CH_4, per day though manure CH_4 yield was greater for PAR
365 ewes than for CON ewes, both per kg DMI ($P<0.05$) and per kg dOMI
366 ($P<0.01$). Likewise, CON and PAR ewes produced similar amount of manure

N, and thus N_2O, per day though N_2O yields were greater for PAR ewes than for CON ewes, both per kg DMI ($P=0.082$) and per kg dOMI ($P<0.05$).

3.5. GHG intensity and sensitivity analysis

Table 4 shows the outcomes of the underlying calculations towards the treatment effects on GHG intensity. PAR and RES ewes required on averaged ~5 days longer than CON ewes to reach the target lamb weaning BW. This was associated with the same total DMI for CON and PAR ewes, but a significantly smaller DMI for RES ewes ($P<0.01$). Total BW loss was greater for PAR ewes than for CON ewes ($P<0.05$), and in turn greater for RES ewes than for PAR ewes ($P<0.05$). The estimated amount of DMI needed to restore BW lost was smaller in CON ewes than in PAR and RES ewes ($P<0.01$). However, the two pools of DMI combined were very similar for CON and RES ewes but greater for PAR ewes ($P<0.05$). The latter was reflected in a greater feed conversion ratio ($P<0.05$). Manure volatile solids and N output were greater for PAR ewes than for CON and RES ewes.

Table 4 also shows that the resulting GHG intensity for each of the underlying sources was greater for PAR ewes than for CON and RES ewes. Combined with the GWP for lucerne production, our calculations show that the GWPs for lamb production of CON, PAR and RES ewes were 5.09, 5.91 and 5.28 kg CO_2-eq per kg lamb weight gain, respectively, which was calculated to correspond with 1.04, 1.24 or 1.10 kg CO_2-eq per kg milk, respectively (s.e.d. 0.04 kg CO_2-eq per kg; $P<0.05$).

The sensitivity analysis indicated that GWP per kg lamb BWG was not very sensitive to variation in urine N excretion; an increase in urine N from

392 0.46 to 0.60 × N absorbed during lactation increased GWP by less than 1.4%
393 from 5.91 to 5.99 CO_2-eq per kg lamb weight gain. However, GWP was more
394 sensitive to variation in ME requirement for ewe BW gain; GWP for PAR ewes
395 varied from 5.69 to 5.91 to 6.13 kg CO_2-eq/kg BWG for ME requirements per
396 kg BW gain taken as 39.75, 49.16 and 58.57 MJ/kg, respectively.
397 Nevertheless, each of these figures were significantly greater than the 5.09 kg
398 CO_2-eq/kg BWG for the CON ewes.
399
400 **4. Discussion**
401
402 Here we propose a framework that accounts for pathogen-induced
403 variation in GHG yield and reduction in feed efficiency in order to
404 experimentally test the hypothesis that periparturient parasitism increases
405 ewe GHG intensity for lamb production. To our knowledge, this is the first set
406 of data that empirically addresses the consequences of impaired animal
407 health on GHG intensity. The data obtained support the view that whilst ovine
408 periparturient gastrointestinal nematode parasitism reduced GHG production
409 per day, it paradoxically resulted in an increased GHG intensity for their lamb
410 BWG by ~16%. Given that the latter was of similar magnitude to the impact of
411 ewe parasitism on feed conversion ratio, the calculated increased GHG
412 intensity largely came from accounting for the impact on production losses
413 rather than on GHG yield per kg dry matter intake (DMI). As such, impact of
414 animal health on GHG intensity is driven by a combination of a reduction in
415 feed intake and feed nutritive value, the latter illustrated by ewe parasitism
416 increasing GHG yield per kg digestible organic matter intake (dOMI).

Periparturient parasitism evoked anorexia in our ewes, especially during lactation, but also reduced milk production and accelerated ewe BW loss, which is in support of earlier observations (Leyva et al., 1982; Zaralis et al., 2009). Although the degree of anorexia was relatively small at 9%, its effect on litter weight gain (milk production) and ewe BW loss would have been exacerbated by the negative impact of parasite challenge on total tract nutrient digestibility, which agrees with other studies (Parkins et al., 1973; Sykes and Coop, 1977). The impact on OM digestibility, which can be seen as a proxy for energy digestibility, resulted in a reduction in dOMI of ~26% for PAR ewes relative to CON ewes. The PAR ewes minimised the impact of the latter on ME availability by mobilising more body fat, as the rate and extent of body condition score loss tended to be greater than in CON ewes. Consequently, it was assumed that the ME requirements per kg BW gain for replenishment of body reserves for PAR ewes upon weaning was also greater than that for CON ewes, due to the higher energy contents of fat (AFRC, 1993). The sensitivity analysis for the arbitrarily taken ME requirement for BW gain of 49.16 MJ/kg as the average of 39.75 MJ/kg for average body composition (AFCR (1993), and 58.57 MJ/kg for fat deposition only (Olthoff et al., 1989), indicated that the impact of ovine parasitism on calculated GHG intensity for lamb weight gain would reduce to 12% if standard assumptions are taken but remains significant, with the largest contribution coming from N_2O. Calculated GHG intensity for lamb weight gain was not very sensitive to urinary N excretion; increasing the latter by up to 40%, i.e. the effect of parasitism on N balance in growing lambs (Parkins et al., 1973; Sykes and Coop, 1977), increased GHG intensity for lamb weight gain by less than 2%.

442 This is reflective of the relatively small proportion of nitrogen intake that is
443 excreted with the urine in lactating ewes, where a significant proportion of
444 dietary nitrogen is excreted with the milk.

445 Compared to our previous studies where similar infection protocols
446 were used in periparturient ewes (Houdijk et al., 2003, Houdijk et al., 2006;
447 Kidane et al., 2010), FEC of the PAR ewes were relatively low with levels
448 below 100 epg. In addition, achieved DMI was relatively high, typically ~60%
449 greater than that of ewes that were fed at 90% of assumed ME requirements
450 (Kidane et al., 2010). The low FEC observed may have been the outcome of
451 concurring high intake levels of metabolizable protein (MP), which is known to
452 reduce ewe FEC through improved host resistance to nematodes (Houdijk et
453 al., 2012). Since *in vitro* OM digestibility (Table 1) was very similar to *in vivo*
454 OM digestibility (Table 2) for CON ewes, we can assume that the reduction in
455 total tract OM and CP digestibility (Table 2) for PAR ewes proportionally
456 reduced book values of fermentable ME and by-pass protein content
457 (Hazzledine, 2014). Consequently, consumed lucerne pellets may have
458 yielded ~70 g MP per kg DMI for PAR ewes, compared to ~80 g MP per kg
459 DMI in CON and RES ewes. This suggests that MP intake for PAR ewes was
460 likely around 330 g per day during lactation. Whilst a similar level of calculated
461 MP intake significantly reduced worm egg output in ewes fed rations based on
462 xylose-treated soya bean meal compared to a low MP control, the same level
463 of calculated MP intake failed to reduce worm egg output in ewes fed rations
464 based on faba beans (Sakkas et al., 2012). Furthermore, the high level of
465 feed intake achieved in our ewes would have resulted in a large weight of
466 faeces, which all else being equal would reduce the FEC due to its expression

as number of eggs per gram and thus its sensitivity to faecal dilution (Houdijk, 2008). Indeed, the calculated total worm egg output of approximately one million eggs per day around day_{28} of lactation was in agreement with that observed when MP supply was scarce, even though FEC were about four times greater than in our PAR ewes (Kidane et al., 2010). Taken together, the above would support the view that immunity to parasites was at least to some extent compromised in our PAR ewes, in contrast to what the low FEC we reported here would intuitively indicate.

Ruminants lose 2 to 12% of their gross energy intake in the form of CH_4 (Johnson and Johnson, 1995), which accounts for a significant proportion of GHG emissions from livestock production systems. In our data, observed CH_4 yield averaged 10.23 g/kg DMI, equivalent to 3.3% of gross energy, which is relatively low compared to CH_4 energy losses on lucerne reported elsewhere, e.g. 4.7% on lucerne hay fed *ad libitum* (Pinares-Patinõ et al., 2003), 5.1% on pelleted lucerne fed restrictedly (Pinares-Patinõ et al., 2013; Waghorn et al., 2002), 5.9% on silage fed *ad libitum* (Bouchard et al., 2013), or 6.6% on freshly-cut and fed *ad libitum* (Waghorn et al., 2002). Furthermore, it was also lower than the 6.3% loss of CH_4 energy and out of the range of 3.7% to 13.3% reported from 61 studies in housed and grazing sheep fed a large variety of forage-based rations (McBride et al., 2013). There may be several reasons why CH_4 yield observed in our study was low. The high level of feed intake achieved in our ewes was likely a major contributor, since it is well established that CH_4 yield decreases with increased feed intake (Johnson and Johnson, 1995; Jentsch et al., 2007), which reduces rumen retention time (AFRC, 1993), and thus reduces CH_4 emissions (Goopy et al., 2014). Intake

492 was likely high since lucerne was offered as dried pellets rather than as long
493 forage. In agreement with this observation, it has been reported that CH_4 yield
494 on grass nuts was ~35% less than on fresh grass or grass silage in lowland
495 replacement ewes (Aubry et al., 2014). In addition, subject to variety,
496 environment and harvest date (Pecetti et al., 2006), lucerne is known to
497 contain variable amounts of saponins, which may not only mitigate
498 methanogenesis (Jayanegara et al., 2010; Cieslak et al., 2013), but may also
499 be anthelmintic (Ali et al., 2011), and it can therefore not be excluded that this
500 may also have contributed to the relatively low FEC observed.

501 In our experiment, observed CO_2 yield averaged 690 g per kg DMI,
502 which agrees with the earlier respiration and carbon balance measurements
503 using sheep, fed restricted amounts of dried grass (Blaxter and Graham,
504 1955; Midwood et al., 1994). Tables 3 and 4 show that breath CO_2 could be
505 considered proportionally the largest component of GHG production, yields
506 and intensities measured. However, since breath CO_2 is an inevitable
507 consequence of metabolism, CO_2 contributions to GWP calculations are those
508 arising from fossil fuel use only (Cederberg et al., 2013). However, even if we
509 include breath CO_2 in our GWP calculations, the relative effect of parasitism
510 on GHG intensity remains 16%. Within the animal derived GHG contributions,
511 enteric CH_4, manure N_2O and manure CH_4 comprise 67, 30 and 3%,
512 respectively for CON and RES ewes and 64, 32 and 4% respectively for PAR
513 ewes in the final calculated GWP per kg lamb BWG figures. However, the
514 relative contribution of these GHG sources to the increase in calculated GHG
515 intensity averaged at 47, 43 and 7%, respectively. Whilst this indicates that
516 enteric CH_4 remains the largest contributor of GWP per kg lamb BWG, enteric

517 CH_4 and manure N_2O contributed almost equally to the parasitism-increased
518 GHG intensity.

519 We identified that the impact of parasitism on observed and calculated
520 yields per kg dOMI was more pronounced than per kg DMI (Table 3).
521 Although this was the direct consequence of impact of parasitism on total tract
522 OM digestibility (Table 2), it raises the question what would be the most
523 appropriate unit to express GHG yield. It might be argued that most studies
524 resort to yield per unit DMI since data on total tract OM digestibility are not
525 generally available. Since it has been suggested that animals aim to optimise
526 ME intake through their feeding behaviour (Tolkamp, 2010), and ME is directly
527 proportional to the digestible organic matter component of the ration, dOMI is
528 an excellent proxy in the absence of respiration data. Furthermore, variation in
529 GHG production is better explained per unit dOMI rather than DMI, as the
530 former better accounts for differences in diet quality (Muetzel, 2009). In this
531 respect, it might be argued that the 23% relative impact of parasitism on GHG
532 intensity on the basis of yield per kg dOMI is not dissimilar to the 16%
533 increase on the basis of GHG intensity per kg lamb BWG. Hence, when OM
534 digestibility data are available, expression of GHG yield per unit dOMI may be
535 preferred over GHG yield per unit DMI, as it would also be more reflective of
536 the most preferred expression, i.e. GHG per unit product (Hristov et al., 2013).

537 The functional unit in our study was one kg of lamb BW gain between
538 birth and weaning, and we used this to estimate the level of milk production
539 (Robinson et al., 1969). Although the aim of our work was not to develop a full
540 life cycle assessment for ewe milk production, the averaged calculated GHG
541 intensity of 1.13±0.02 kg CO_2-eq per kg milk fitted in the wide range of

intensities reported for sheep milk production from earlier life cycle assessments, i.e. from 0.9-1.7 kg CO_2-eq per kg milk (Haas et al., 2001) but was lower than more recent estimates, i.e. 1.8-4.5 kg CO_2-eq per kg milk (Batalla et al., 2004) and 8.4 CO_2-eq per kg milk (Opio et al., 2013). This likely reflects the development in life cycle assessment methodologies over the last few years, and an increased understanding of GHG sources that require to be taken into account (Cederberg et al., 2013). In the context of controlling parasitism, this would need to include GHG emmisions arising from the intervention.

Using the reported impacts of parasitism on FCR and assuming no impact on GHG yields, it can be calculated that lamb parasitism may increase GHG intensities to greater magnitudes than observed here in ewes, by up to 21% under housed conditions (Kyriazakis et al., 1994; Zaralis et al., 2008) and up to 139% under field conditions (Thamsborg and Agergaard, 2002). These figures could increase significantly if lamb parasitism does increase GHG yield, which has yet to be established. Thus, improvement of animal health has great potential to contribute to climate change mitigation strategies (Shields and Orme-Evans, 2015). Indeed, a 10% reduction in calculated GHG intensity has been estimated from suppressive anthelmintic treatments in continuously naturally infected, growing lambs (Keynon et al., 2013), though in agreement with our observations, reduced exposure can be expected to result in an even greater performance benefit (Coop et al., 1982) and thus reduction in GHG intensity. However, studies that have examined implications of improving animal health on GHG emission intensity are scarce, despite that improving feed efficiency has been recognised as a major driver to reduce

GHG emissions (Basarab et al., 2013), and connections among animal health and resource efficiency are obvious (Hristov et al., 2013). Perhaps as a consequence, improving animal health as a climate change mitigation option is not often considered amongst other more technical options, including reducing undernourishment (Eckard et al., 2010; Gerber et al., 2013). The framework developed here and the results for the specific hypothesis tested, may provide a tool and impetus for further studies in this field, including assessing different nutritional and challenge environments. Furthermore, they may assist to ensure that improvement of animal health is an increasingly recognised and integrated component of efforts to reduce the environmental footprint of animal production systems.

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Legends to figures

Figure 1. Dry matter intake of twin-rearing ewes fed pelleted lucerne, either sham-infected and fed *ad libitum* (○), or trickle-infected with *Teladorsagia circumcincta* and fed *ad libitum* (●), or sham-infected and fed restrictedly during lactation at 80% of intake by ewes fed *ad libitum* (◊).

Figure 2. Hourly methane and carbon dioxide production of pairs of pelleted lucerne fed twin-rearing ewes at 5 wks into lactation, either sham-infected and fed *ad libitum* (○), or trickle-infected with *Teladorsagia circumcincta* and fed *ad libitum* (●), or sham-infected and fed restrictedly during lactation at 80% of intake by ewes fed *ad libitum* (◊). The arrow indicates time of feeding.

820 Table 1. Analysed composition of lucerne.

821

Analysis	
Dry matter (g/kg)	974
Neutral detergent fibre (g/kg DM)	448
Acid detergent fibre (g/kg DM)	362
Crude protein (6.25×N, g/kg DM)	163
Ash (g/kg DM)	103
Acid hydrolysis ether extract (AH-EE, g/kg DM)	19.4
Acid insoluble ash (g/kg DM)	13.7
In vitro organic matter digestibility (NCGD[1], %)	57.2
Gross energy (MJ/kg DM)	17.9
Digestible energy[2] (MJ/kg DM)	10.2
Metabolizable energy[3] (MJ/kg DM)	8.3

822

823 [1]Neutral cellulose and gammanase digestibility

824 [2]Calculated as ME/0.81 (AFRC, 1993)

825 [3]Calculated from AH-EE and NCGD (Thomas et al., 1988).
826

827 Table 2. Apparent feed digestibility and ewe performance during lactation of
828 twin-rearing ewes fed pelleted lucerne, either sham-infected and fed *ad*
829 *libitum* (CON), or trickle-infected with *Teladorsagia circumcincta* and fed *ad*
830 *libitum* (PAR), or sham-infected and fed restrictedly during lactation at 80% of
831 intake by ewes fed *ad libitum* (RES).

832

	Treatments[1]			
	CON	PAR	RES	s.e.d.
Apparent digestibility				
Dry matter (DM, %)	53.8[a]	45.6[b]	55.3[a]	2.98
Organic matter (OM, %)	54.6[a]	46.4[b]	55.8[a]	2.99
Crude protein (CP, %)	58.8[a]	53.4[b]	61.9[a]	2.87
Performance				
DM intake (kg/day)	4.58[a]	4.29[b]	3.55[c]	0.10
Digestible OM intake (kg/day)	2.24[a]	1.78[b]	1.77[b]	0.13
Digestible CP intake (kg/day)	0.44[a]	0.37[b]	0.36[b]	0.02
Ewe body weight gain (g/day)	-69[a]	-162[b]	-252[c]	37
Litter body weight gain (g/day)	718[a]	669[b]	657[b]	24
Estimated milk production (g/day)	3524[a]	3223[b]	3142[b]	149

833
834 [1]Data within the same row with different superscripts differ (P<0.05).

Table 3. Observed and calculated greenhouse gas production and yield during lactation of twin-rearing ewes fed pelleted lucerne, either sham-infected and fed *ad libitum* (CON), or trickle-infected with *Teladorsagia circumcincta* and fed *ad libitum* (PAR), or sham-infected and fed restrictedly during lactation at 80% of intake by ewes fed *ad libitum* (RES).

	Treatments[1]			
	CON	PAR	RES	s.e.d.
Enteric methane (observed)				
CH_4 production (g/day/ewe)	55^{a}	49^{b}	41^{c}	2.8
(CO_2-eq)	1375^{a}	1225^{b}	1025^{c}	70
CH_4 yield (g/kg DMI)	10.6	10.3	10.3	0.56
(CO_2-eq)	265	258	258	14
CH_4 yield (g/kg dOMI)	22.0^{a}	25.2^{b}	20.9^{a}	1.54
(CO_2-eq)	550^{a}	630^{b}	523^{a}	39
Manure methane (calculated)				
Volatile solids production (kg/day)	2.24^{a}	2.44^{a}	1.69^{b}	0.12
CH_4 production (g/day/ewe)	2.86^{a}	3.10^{a}	2.15^{b}	0.14
(CO_2-eq)	72^{a}	78^{a}	54^{b}	4
CH_4 yield (g/kg DMI)	0.56^{a}	0.65^{b}	0.54^{a}	0.03
(CO_2-eq)	14^{a}	16^{b}	14^{a}	1
CH_4 yield (g/kg dOMI)	1.22^{a}	1.79^{b}	1.15^{a}	0.24
(CO_2-eq)	30^{a}	45^{b}	29^{a}	6
Manure nitrous oxide (calculated)				
N excretion (g/day)	91.6^{a}	89.6^{a}	68.7^{b}	2.77
N_2O production (g/day/ewe)	2.05^{a}	2.01^{a}	1.54^{b}	0.06
(CO_2-eq)	611	599	459	18
N_2O yield (g/kg DMI)	0.40^{ab}	0.42^{a}	0.39^{b}	0.01
(CO_2-eq)	119^{ab}	125^{a}	116^{b}	3
N_2O yield (g/kg dOMI)	0.85^{a}	1.12^{b}	0.81^{a}	0.12
(CO_2-eq)	253^{a}	334^{b}	241^{a}	36
Methane and nitrous oxide combined as CO_2-eq (calculated)				
GHG production (g/day/ewe)	2044^{a,x}	1909^{a,y}	1524^{b}	72
GHG yield (g/kg DMI)	399	397	386	14
GHG yield (g/kg dOMI)	844^{a}	1039^{b}	798^{a}	83
Breath carbon dioxide (observed)				
CO_2 production (g/day/ewe)	3463^{a}	3241^{a}	2844^{b}	147
CO_2 yield (g/kg DMI)	676	674	721	26
CO_2 yield (g/kg dOMI)	1413^{a}	1664^{b}	1470^{ab}	111

[1]Data with different superscripts differ ($^{a,b,c}P<0.05$; $^{x,y}P<0.10$).

841 Table 4. Effects of maternal parasitism and restricted feeding on time and dry
842 matter intake (DMI) required to wean two lambs at 25 kg each and
843 compensate for loss of body weight (BW), and its effect on calculated ewe
844 global warming potential (GWP) of twin-rearing ewes fed pelleted lucerne,
845 either sham-infected and fed *ad libitum* (CON), or trickle-infected with
846 *Teladorsagia circumcincta* and fed *ad libitum* (PAR), or sham-infected and fed
847 restrictedly during lactation at 80% of intake by ewes fed *ad libitum* (RES).
848

		Treatments[1]			
		CON	PAR	RES	s.e.d.
Calculated performance and manure production parameters					
Days to target (n)		57.1[a]	61.8[b]	62.7[b]	2.39
Total BW lost (kg)		3.4[a]	10.0[b]	14.4[c]	1.85
DMI to target (kg)		253[a]	257[a]	214[b]	9.5
DMI to compensate BW loss (kg)		16[a]	60[b]	68[b]	9.5
Total DMI (kg)		270[a]	316[b]	283[a]	11.6
Feed conversion ratio[2]		6.7[a]	7.8[b]	7.0[a]	0.31
Manure volatile solids (kg)		115[a]	151[b]	118[a]	10.2
Manure N output (kg)		4.75[a]	6.11[b]	5.10[a]	0.28
Calculated GWP (kg CO_2-eq/kg lamb body weight gain)					
Enteric	CH_4	1.79[a]	2.00[b]	1.80[a]	0.10
Manure	N_2O	0.80[a]	1.00[b]	0.87[a]	0.05
	CH_4	0.09[a]	0.12[b]	0.10[a]	0.01
Feed	CO_2-eq	2.41[a]	2.79[b]	2.51[a]	0.11
Total GWP		5.09[a]	5.91[b]	5.28[a]	0.21
Calculated breath CO_2 (g/kg lamb body weight gain)					
		4.52[a]	5.21[b]	4.96[b]	0.19

849
850 [1]Data within the same row with different superscripts differ ([a,b,c]P<0.05).
851 [2]Feed conversion ratio is calculated as total DMI divided over total lamb BW
852 gain.
853
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